Industrial Scale-Up of Countercurrent Chromatography: Predictive Scale-Up

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Abstract

This study describes how scale-up in countercurrent chromatography (CCC) can be simply predicted on a process scale CCC device by running a preliminary analytical-sized sample and having knowledge of the stationary-phase retention at scale-up conditions. Results have shown that simple experimentation can lead within a day to a process with the capability of several kilograms per day (tons per year) compound yield, and that this is feasible with benchtop CCC units.

Introduction

There are few processes that can be scaled up from laboratory to production scale without difficulties. For example, preparative high-performance liquid chromatography (HPLC) is not a linear scale-up, is expensive, and uses large volumes of solvents. The product can become hydrolyzed by or react with the column, can induce chemical/steric/chiral conformation changes, and often requires significant prepurification with further risk of degradation.

Countercurrent chromatography (CCC) (1,2) is a process that avoids these difficulties. It is a form of liquid–liquid chromatography without a solid support that separates soluble substances such as natural products on their partition or differential solubility between two immiscible solvents. The principle of separation (partition) is the same in both the laboratory and production plant and is generic in that it can be applied to an extremely broad range of purification problems in many industries. Furthermore, because there is no solid support, there is 100% sample recovery and no need for any prepurification.

The operational process is extremely simple. It can be considered as consisting of a sample, a length of tubing, and two immiscible solvent phases. The tubing is initially filled with the solvent intended to be the stationary phase, and the sample is injected with the mobile phase. After an appropriate period of time, fractions of the injected sample emerge from the downstream end of the tubing in the order of their partition coefficients. The tubing, usually poly(tetrafluoroethylene), is wound on a drum, which is centrifugally rotated in planetary motion. This sets up alternating zones of mixing and settling along the length of the tube synchronous with the high and low "g" sides of the coil. Samples injected with the mobile phase undergo as many as 100,000 partitioning steps per hour, resulting in high-resolution separations with no sample adsorption onto solid supports. The mixing efficiency is excellent, and the process is not limited by hydrostatic pressure.

The planetary motion and hardware used in this study have been described in an earlier study (3). Scale-up modifications to the hardware will be described in the Methodology subsection. The present study demonstrates the versatility and potential of CCC by showing that scale-up is predictable and can be analyzed theoretically once a basic low-sample-volume chromatogram has been obtained and stationary-phase retention is known. Furthermore, because of the linear relationship between the retention and the square root of flow (4,5), only two retention values at different flows at a given rotational speed need to be obtained before a complete process scale scenario can be planned.

Experimental

Theory

Retention

Before retention can be correctly measured, it is necessary to know the coil system volume (V_c) and the volume of the inlet, link, and outlet flying leads (V_{in}, V_{link}, and V_{out}, respectively). These can be measured gravimetrically or calculated from the bore and the length of the tubing.

The coil system is initially filled with the intended stationary phase and the pump/sample injection system primed with the intended mobile phase. With the rotor speed and

direction set, the pump is then activated with a low flow (initially) and the eluent collected in a fresh measuring cylinder. The volume of the stationary phase eluted (V_e) is measured as follows:

$$V_e = V_m + V_{in} + V_{link} + V_{out} \quad \text{Eq. 1}$$

thus the mobile phase volume (V_m) can be found:

$$V_m = V_e - (V_{in} + V_{link} + V_{out}) \quad \text{Eq. 2}$$

The volume of the stationary phase retained in the coil (V_s) can then be obtained by subtracting V_m from V_c:

$$V_s = V_c - V_m \text{ or } V_m = V_c - V_s \quad \text{Eq. 3}$$

The stationary phase retention (S_f) is generally expressed as a percentage of V_c as follows:

$$S_f = 100V_s/V_c \quad \text{Eq. 4}$$

Resolution

In CCC as in most other forms of liquid chromatography, the partition coefficient (K) is generally described using the concentration of solute in the stationary phase (C_s) and the concentration of solute in the mobile phase (C_m) by the following equation:

$$K = C_s/C_m \quad \text{Eq. 5}$$

Occasionally, as with countercurrent distribution, K can be expressed as the reciprocal of this value.

In column chromatography, the most common term used is the retention factor (k'), which is related to K by the phase volume ratio of the quantity of solute in the stationary phase (Q_s) and the quantity of solute in the mobile phase (Q_m):

$$k' = Q_s/Q_m = C_sV_s/C_mV_m = K(V_s/V_m) = KS_f/(100 - S_f) \quad \text{Eq. 6}$$

Conway (6) has described how both K and the retention ratio ($k' = t_k'/t_m$, in which t_k' is the time from the solvent front to the elution of the K peak and t_m is the time from the sample injection to the solvent front) relate to the CCC chromatogram in Figure 1. However, for this study only K will be used:

$$K = t_k'/t_c' = (t_k - t_m) / (t_c - t_m) \quad \text{Eq. 7}$$

$$t_k = t_m + K(t_c - t_m) = t_m + Kt_c' \quad \text{Eq. 8}$$

where t_c' is the time from the solvent front to the elution of the peak at which K = 1, t_k is the time from the sample injection to the elution of the K peak, and t_c is the time from the sample injection to the elution of the peak at which K = 1.

In order to take measurements from the chromatogram, it is important to know where both the solvent front (i.e., K = 0) and the peak for which K = 1 elutes. The solvent front can be derived from equations 3 and 4 as follows:

$$t_m = V_m/F = (V_c - V_s)/F = V_c/F - V_cS_f/100F = (V_c/F)(100 - S_f)/100 \quad \text{Eq. 9}$$

where F is the mobile phase flow rate.

The point at which K = 1 is where the sample is equally soluble in both phases. Therefore, the phase system behaves like a single phase, and the component elution at which K = 1 coincides with the system volume (V_c), or in time terms:

$$t_c = V_c/F \quad \text{Eq. 10}$$

Figure 1 shows the effect of increasing the sample volume (V_i). If the normal peak width from a low-volume sample (V_o) is w_k, then it will become $w_k + t_i$ (in which $t_i = V_i/F$) when there is an increase in the sample volume, provided that the elution concentrations are similar to the injection concentration and the sample does not have a surfactant effect on the phase system causing further elution of the stationary phase. It should be noted that V_o is extremely small with respect to V_i and can be ignored.

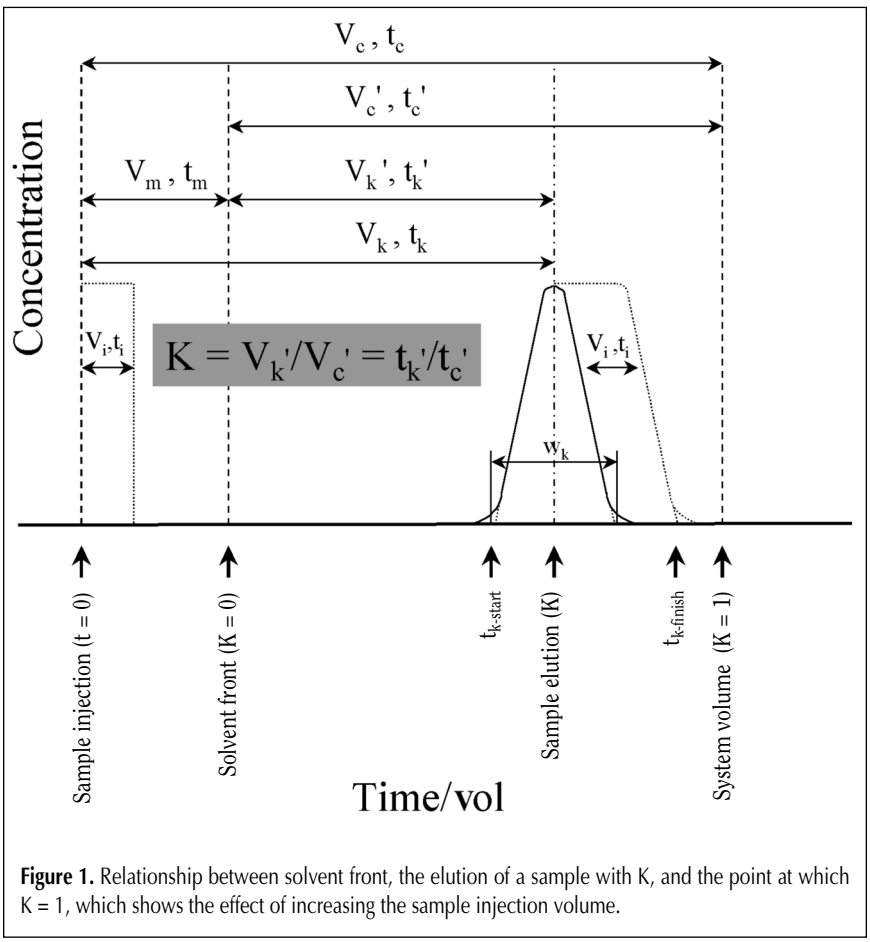

Figure 1. Relationship between solvent front, the elution of a sample with K, and the point at which K = 1, which shows the effect of increasing the sample injection volume.

If the peak width or degree of diffusion is known from an analytical sample experiment, then the beginning and end of the sample elution for larger volume injections can be calculated with the previously mentioned assumptions as follows:

$$t_{k\text{-start}} = t_k - w_k/2 \qquad \text{Eq. 11}$$

$$t_{k\text{-finish}} = t_k + w_k/2 + V_i/F \qquad \text{Eq. 12}$$

Throughput can then be maximized by increasing the sample volume injected until peaks start to overlap. In a two-component system with partition coefficients K_1 and K_2, the maximum sample injection can be calculated by using equations 11 and 12 so that the finish of the first peak coincides with the start of the second (as illustrated in Figure 2):

$$t_{k1\text{-finish}} = t_{k2\text{-start}} \qquad \text{Eq. 13}$$

$$t_{k1} + w_{k1}/2 + V_i/F = t_{k2} - w_{k2}/2 \qquad \text{Eq. 14}$$

$$t_i = V_i/F = (t_{k2} - t_{k1}) - (w_{k1} + w_{k2})/2 = \Delta t(R_s - 1)/R_s \qquad \text{Eq. 15}$$

where R_s (the resolution between two peaks) is found by:

$$R_s = 2(t_{k2} - t_{k1}) / (w_{k1} + w_{k2}) = 2\Delta t/(w_{k1} + w_{k2}) \qquad \text{Eq. 16}$$

R_s clearly has to be greater than unity for any possible increase in sample volume. The maximum sample injection volume for a dual peak system can therefore be calculated from measurements of peak widths and peak separation taken from a low-volume injection chromatogram.

The total elution time (t_{tot}) is given by:

$$t_{tot} = t_{k2\text{-finish}} - t_{k1\text{-start}} \qquad \text{Eq. 17}$$

The maximum throughput (V_{max}) is achieved by timing serial sample injections so that the final elution of peak 2 coincides with the initial elution of peak 1 from the next sample injection and so on:

$$V_{max} = V_i/t_{tot} \qquad \text{Eq. 18}$$

The maximum mass throughput per unit time can be obtained by multiplying the maximum volumetric throughput per unit time (V_{max}) by the maximum possible sample concentration.

Methodology

Apparatus

Chromatograms were performed using a modified temperature-controlled Quattro coil planet centrifuge (3) supplied by Romulus Technology (Brunel University, Uxbridge, U.K.) as part of a BBSRC/DTI LINK program on the industrial scale-up of CCC. The rotor radius was 11 cm. There were two bobbins (β range from 0.63 to 0.87) connected in series with one coil on each bobbin. Each coil consisted of 84 loops of 3.68-mm-i.d. stainless steel tubing with a defined pitch of 5.5 mm and a capacity of 464 mL. The total capacity was 928 mL with speed varying from 0 to 1400 rpm and flow from 0 to 100 mL/min. The chromatography set-up was comprised of a Gilson HPLC pump (Model 302) with a 100SC head (Anachem, Luton, U.K.), a Grant (Y6) waterbath for preheating the mobile phase to 30°C (Grant Instruments Cambridge Ltd., Herts, U.K.), a Rheodyne (Cotati, CA) sample valve with a 100-mL sample loop, a Cecil (Cambridge, U.K.) UV spectrophotometer (type CE272) set to 262 nm, and a Gould (Model BS272) chart recorder (Servoscript Instrument Services, Berks, U.K.). The rotor cabinet was maintained at 30°C ± 2°C. A Grant cooler (type RC1400G) supplied water–glycol coolant for the temperature control system (Grant Instruments Cambridge Ltd.).

The end of the tubing to which a bubble or bead would move under the action of Archimedean screw action was termed "Head", and the opposite end of the tubing to the "Head" was the "Tail". The outside of the coil of tubing with the highest β value was termed the "Periphery", and the inside of the coil of tubing with the lowest β value was the "Center".

Forward rotation was "Head" center "Tail" periphery. Reverse rotation was "Tail" center "Head" periphery. With the lower phase mobile, the rotation was "Forward" with the inlet arranged to be from "Head" center to "Tail" periphery in order

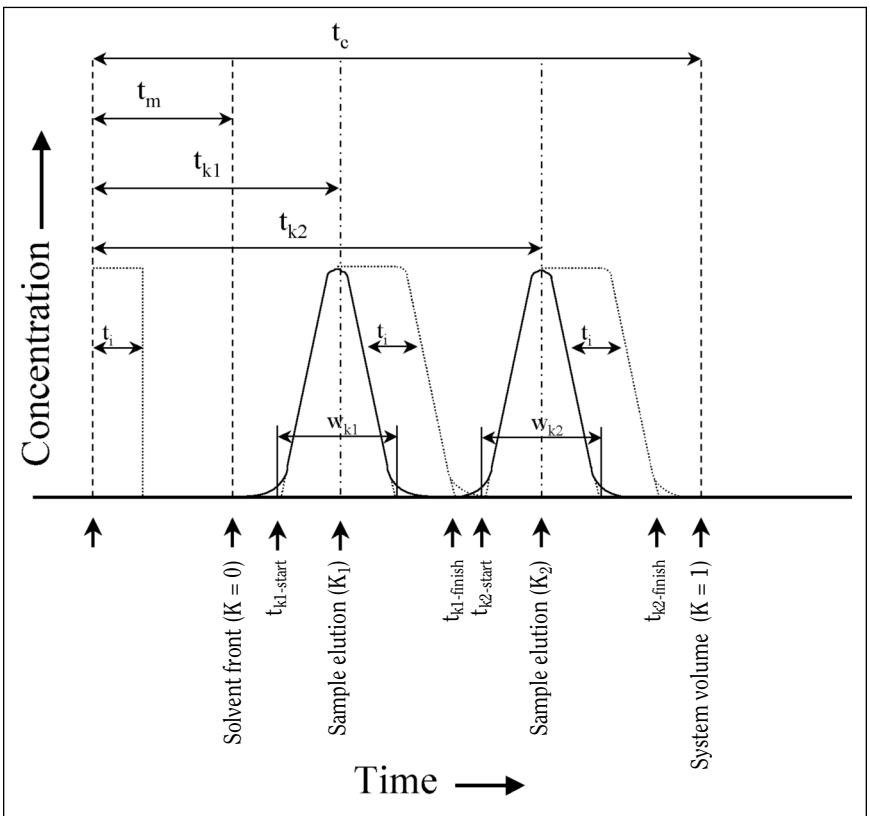

Figure 2. Relationship between solvent front, the elution of a sample with K_1 and K_2, and the point at which K = 1, which shows the effect of increasing the sample injection volume.

to keep the Archimedean and hydrostatic forces additive and working together (7).

Phase system

The phase system (abbreviation 4A) was made up of heptane, ethyl acetate, methanol, and water (1.4:0.1:0.5:1.0) with the constituent solvents supplied by Merck BDH (Merck UK Ltd., Lutterworth, U.K.). Six liters were prepared, mixed, and allowed to equilibrate for 24 h in advance of an experimental run. The equilibrated phase system was out gassed by sonication for 20 min using a Jencon Soniclean (Jencons Scientific Ltd., Beds, U.K.).

Sample mixture

An idealized sample mixture containing 4% solutions of both benzyl alcohol (C_7H_8O – K = 0.28) and 2-phenyl-ethanol ($C_8H_{10}O$ – K = 0.46) was supplied by Sigma Chemicals Co. (St. Louis, MO). The separation factor (α) was 1.64. One milliliter each of benzyl alcohol and 2-phenyl-ethanol were increased to 25 mL with a lower phase from the phase system 4A described previously. The sample volumes used in this study were 2, 25, and 100 mL, which would be equivalent to 80 mg, 1 g, and 4g, respectively.

Retention tests

Assuming the CCC coils have been purged with nitrogen, the Gilson pump, sample loop, and waterbath preheating coil were first filled with upper phase (which was intended to be the stationary phase) by switching the V1 control valve in Figure 3 to "upper phase" and the V2 control valve to "purge". V2 was then switched to "run", the outlet tube placed in a measuring cylinder, and the pump activated to fill the CCC coils with the upper phase at 80 mL/min. When the upper phase eluted, the CCC was rotated in "Reverse" at 300 rpm for a short time in order to pump out any air from the outlet (the "Head" or "Periphery" when in reverse). The pump and rotor were then stopped.

The Gilson pump, the sample loop, and the waterbath preheating coil were then primed with "lower phase" with V1 switched to "lower phase" and V2 switched to "purge". Once primed, V2 was switched to "run" and the outlet tube secured in place in an empty 500-mL measuring cylinder. The desired rotor speed was then set in the forward direction, the control temperature set to 30°C, and the pump set to 10 mL/min. The pump and stopwatch were then simultaneously activated. The logging time and volume eluted gave a check on the actual flow compared with the set flow. Any reduction would signify a pump fault or a leak.

Initially, the upper (stationary) phase was eluted from the outlet tube. When the lower (mobile) phase broke through, the lower phase continued to be pumped until the volume of the upper phase displaced became constant—the system was then in equilibrium. At a convenient time, the pump and stopwatch were stopped simultaneously and the total- and lower-phase volumes noted, leaving the CCC bobbins still rotating. A subtraction of the two gave the volume of the stationary phase eluted, from which the retention could be calculated (see equations 1–4). It should be noted that stopping the pump when the rotor is still rotating will redistribute the phases in the coil (the heavier, or lower, phase moving to the "Tail" and the lighter, or upper, phase moving to the "Head"). A new hydrodynamic equilibrium could then be established at a higher flow by setting the pump to 20 mL/min and restarting the pump and stopwatch. After a short initial period of mobile phase elution, more stationary phase was eluted until a new hydrodynamic equilibrium was reached. Again, the pump and stopwatch were stopped simultaneously after a suitable elution period and the new total- and lower-phase levels noted from which the new stationary phase volume and retention could be calculated. A similar procedure was used for 40 mL/min and 80 mL/min, although fresh measuring cylinders were needed after each flow setting. Once these retention variations with flow tests were completed, the rotor, pump, and stopwatch were simultaneously switched off. Once the rotor was stationary, a nitrogen line was connected to the inlet via valve V3 in Figure 3, the pressure set on the nitrogen regulator to 4 bar, and the contents pumped out of the coils into a 1-L measuring cylinder. Only approximately 800 mL could be eluted in this way. When nitrogen eluted from the outlet, the rotor was set to rotate at 300–500 rpm in reverse in order to screw any retained phase to the outlet tube, which then became the "Head". It was checked that at least 900 mL was eluted, although there may have been some losses because of evaporation and some residue liquid left in the coils.

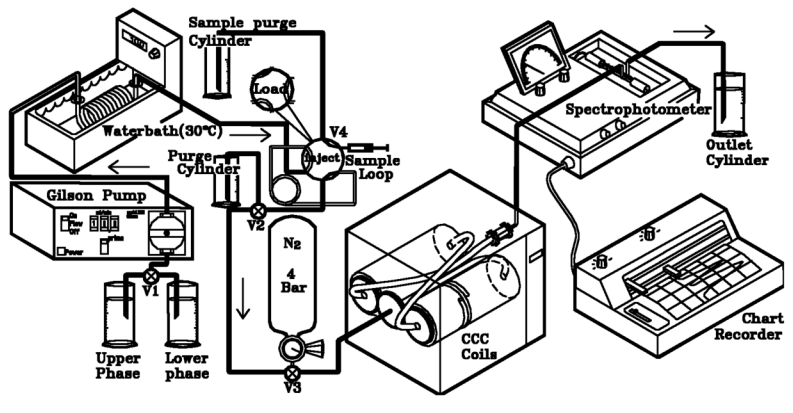

Figure 3. Schematic diagram of a CCC operating system.

Resolution tests

The retention procedure was also used for resolution tests. The main difference was that the outlet tube was connected to the Cecil spectrophotometer, which was equipped with a Helma flowthrough cell with a 1-cm pathlength.

Mobile phase flow was initiated once the rotor speed was set to its desired value. A sample was injected into the sample loop only after hydrodynamic equilibrium was reached (with elution of mobile phase). The pump was briefly stopped when the sample valve V4 in Figure 3 was switched from "Load" to "Inject". For high flows, the flow

was split 1:5 between the spectrophotometer and a bypass into a collection chamber.

Fraction analysis

Fractions were analyzed using an HPLC column (Hichrom Spherisorb ODS 2, 5 µm, 250 × 4.6 mm). The mobile phase was water–acetonitrile (65:35) (HPLC grade; purchased from Fisher Chemicals, Loughborough, U.K.). The flow rate was 1.5 mL/min, the temperature ambient, and the HPLC detection wavelength 254 nm.

Results and Discussion

Predicting retention from linear regressions of flow retention data

The retention of phase system 4A was measured for flows of 10, 20, 40, and 80 mL/min at rotational speeds of 800 and 1200 rpm and plotted in Figure 4 against the square root of flow. Following the example of Du et al. (4), linear regressions at both speeds gave high correlation coefficients (R^2 = 0.994 at 800 rpm and R^2 = 0.999 at 1200 rpm), which allowed for a confident prediction of retention at other flow rates not tested. If an intermediate speed was chosen (i.e., 1000 rpm), then its retention versus $F^{1/2}$ linear regression could be interpolated from those in Figure 4 by taking the average of the linear regression equations.

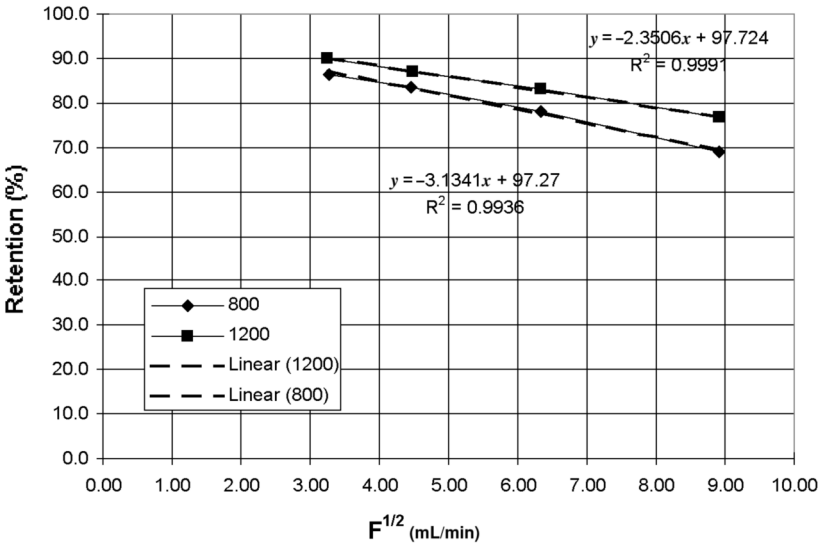

Figure 4. Variation of retention with the square root of flow using the high-speed process scale CCC (1000-rpm retention can be interpolated as $y = -2.7423x + 97.497$).

Predicting the time from sample injection to solvent front elution

Once the stationary phase retention at a given flow and speed was known, the volume of mobile phase and the time for the elution of the solvent front could be calculated from equation 9 knowing the coil volume.

Predicting peak elution times

With the solvent front elution time calculated and knowing the K = 1 elution point from equation 10 and the partition coefficients of the two samples (K_1 and K_2), it was then possible to calculate the time for the elution of the two sample peaks (t_k = 0.28 and t_k = 0.46) from equation 8.

Predicting peak widths

Although the central peak position could be determined from knowledge of the retention, flow, and partition coefficient, the peak width depended on the efficiency of the separation process. Poor mixing and separation will lead to broad peaks with low resolution, and good mixing and settling will lead to narrow peaks and high resolution. It can be shown empirically in CCC that peak width is inversely related to flow rate and not significantly affected by rotational speed. It was therefore only necessary to measure peak width at two different flow rates and interpolate peak widths at the other flows. This is illustrated in Table I in which the retention, solvent front, and peak positions (t_{k1} and t_{k2}) have been obtained as described previously and the peak widths from the measurement (in boldface) of analytical chromatograms taken at flows of 20 mL/min and 80 mL/min. Peak width values at other flows were

Table I. Throughput Prediction at 1000 rpm

Flow (mL/min)	Retention (%)	Solvent front (min)	1st peak (min)	1st width (min)	2nd peak (min)	2nd width (min)	Resolution	Maximum sample (mL)	Throughput (mL/h)	Throughput (kg/day)
20	85.2	6.9	17.9	**2.80**	25.0	**3.20**	2.37	82.37	347.14	0.83
40	80.2	4.6	9.8	1.59	13.2	1.83	1.96	65.47	586.80	1.41
80	73.0	3.1	5.5	**0.90**	7.0	**1.05**	1.56	43.89	864.14	2.07
120	67.5	2.5	4.0	0.65	4.9	0.76	1.34	28.44	908.76	2.18
160	62.8	2.2	3.2	0.51	3.8	0.60	1.18	15.96	730.40	1.75
320	48.4	1.5	1.9	0.29	2.1	0.34	0.80	–20.51	–2433.91	–5.84
480	37.4	1.2	1.4	0.21	1.5	0.25	0.57			
640	28.1	1.0	1.2	0.16	1.2	0.20	0.41			

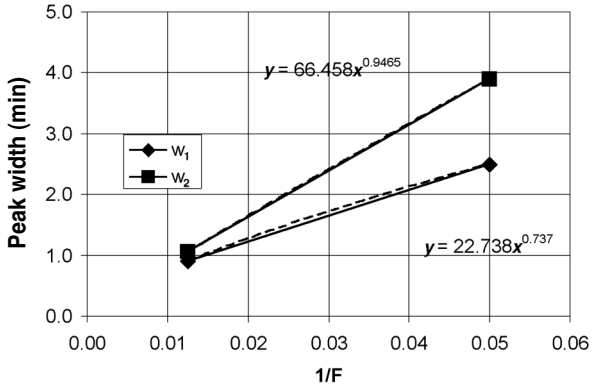

Figure 5. Example of peak width against the inverse of flow for w_1 and w_2 at flows of 20 and 80 mL/min for 1200 rpm (these equations and the appropriate equations for 800 rpm were used to extrapolate peak widths for higher flows).

then interpolated from the best-fit power curve taken from plotting the peak width against the inverse of flow (Figure 5), assuming that peak width tends to zero as flow tends to infinity.

Predicting the start and end of eluted peaks

With the peak width known, it was then possible to predict the beginning and end of each peak elution profile from equations 11 and 12. The predicted start and finish of eluted peaks were compared with measured values (found in Table II) for different flow rates and sample injection sizes. The top half of the table compares predicted and measured peak positions for the chromatogram illustrated in Figure 6 in which the CCC instrument was operating at a speed of 1000 rpm and flow of 20 mL/min. The bottom half of Table II compares predicted and measured peak positions for 80-mL/min flows. Errors were generally within ± 5% at 20 mL/min and ± 7% at 80 mL/min.

Predicting crossover when peaks were not resolved

The predicted values of $t_{k1\text{-finish}}$ and $t_{k2\text{-start}}$ in Table II at 20 mL/min with a sample injection size of 100 mL can be used to help predict how much crossover there was between the two target peaks. The finish of the elution of the first peak ($t_{k1\text{-finish}}$) is predicted to be at 24.3 min, and the prediction of the start of the second peak ($t_{k2\text{-start}}$) is at 23.4 min. If fractions are collected every minute, then there will be predictable mixtures of the two substances in fractions 23 and 24 and fractions 24 and 25. The proportion of each substance in each fraction was predicted and then plotted in Figure 7 (solid line). The fractions were independently analyzed by an HPLC column (AstraZeneca, Hurdsfield, U.K.) and the actual proportions of each substance in each fraction plotted on the same graph (dotted). The correlation between the two is approximately one fraction displaced, which is within ± 5%. This is similar to the errors encountered in determining the positions of the start and finish of peaks in Table II.

Table II. Prediction of the Start and Finish of Each Eluted Peak

	Run	Flow (mL/min)	V_i (mL)	$t_{k1\text{-start}}$ (min)	$t_{k1\text{-finish}}$ (min)	$t_{k2\text{-start}}$ (min)	$t_{k2\text{-finish}}$ (min)
Measured	P9-1	20	2	16.8	19.6	23.5	26.7
Calculated		20	2	16.5	19.3	23.4	26.6
Error (%)				1.7	1.4	0.2	0.2
Measured	P9-2	20	25	15.9	20.5	22.4	29.1
Calculated		20	25	16.5	20.6	23.4	27.9
Error (%)				–3.8	–0.2	–4.5	4.3
Measured	P9-3	20	100	16.4			33.3
Calculated		20	100	16.5	24.3	23.4	31.6
Error (%)				–0.8			5.2
Measured	P9-4	80	2	4.7	5.6	6.2	7.3
Calculated		80	2	5.1	6.0	6.5	7.6
Error (%)				–7.0	–6.0	–4.7	–3.6
Measured	P9-5	80	25	5.0			8.3
Calculated		80	25	5.1	6.3	6.5	7.9
Error (%)				–2.1			5.3
Measured	P9-6	80	100	5.5			
Calculated		80	100	5.5	6.8	7.0	8.3
Error (%)				–1.0			

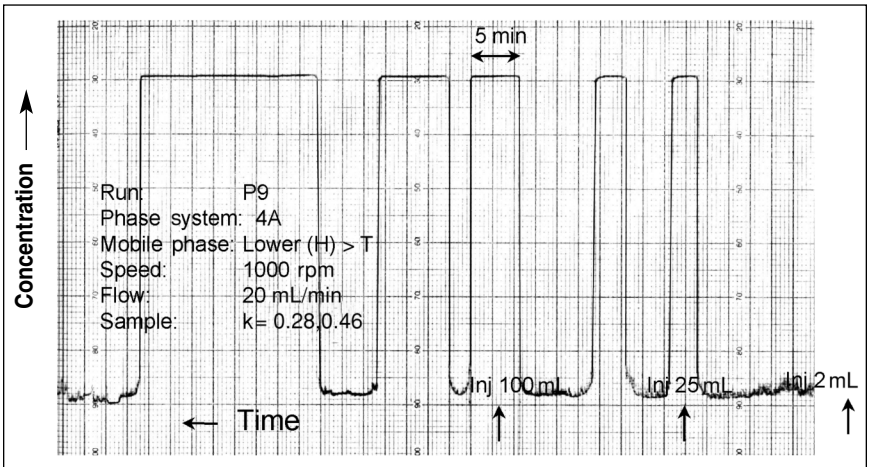

Figure 6. The effect of sample size on resolution.

Predicting the optimum conditions for maximum throughput

This prediction was obtained by calculating the maximum sample injection volume from equations 13–16 and computing the resulting maximum throughput. An example of this is given for 1000 rpm in the last three columns of Table I. Figure 8 shows the variation of predicted throughput with flow for various speeds. The maximum predicted throughput is summarized in Table III for each of the three speeds evaluated. It can be seen that as rotational speed increases, it is possible to maintain a given retention at increasing flow rates and thus

obtain a higher throughput. In this idealized example, with a high interfacial tension phase system, high sample solubility, reasonable retention characteristics, and a separation factor of 1.64, it is possible to achieve sample throughputs of 2.72 kg/day, which if maintained could lead to a throughput of one ton per year from a benchtop device.

Conclusion

The results presented illustrate that it is possible to predict peak elution profiles and make predictions for maximizing sample throughput to within ± 5% accuracy. From these results, it is possible to calculate when peaks will elute. It is also possible to calculate the optimum time to inject a follow-up sample by arranging for the elution of the last component of the previous injection to coincide with the first component of the next. Furthermore, it is possible to extrapolate from this theory in order to predict the throughput for much higher flows at lower retentions.

In a production scenario, it is important to be able to respond quickly to new product demands. The retention tests shown in Figure 4 and the two analytical chromatograms at 20 and 80 mL/min were completed within a day. From these test results, it was possible to find the optimum throughput scenario at 160 mL/min for 1200 rpm. This predictive approach will also allow cost scenarios to be built in. For example, if solvent usage is taken into account, it can be concluded from Figure 8 that there would be only a marginal decrease in throughput for a considerable reduction in flow from 160 mL/min to 100 mL/min.

This preliminary study is limited to a simple two-component sample. In a real system, there may be more components, depending on whether the CCC is being used as a primary or secondary separation process. Either way, partition coefficients can be calculated from preliminary chromatograms obtained with low-volume sample injections (as illustrated) and scale-up scenarios investigated without spending an enormous amount of time undertaking test work.

Currently work is in progress for applying this predictive scale-up approach to a particular antibiotic pilot production process.

Acknowledgments

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Table III. Variation of Maximum Throughput with the Speed of Rotation

Speed (rpm)	Retention (%)	Optimum flow (mL/min)	Maximum throughput (mL/h)	Maximum throughput (kg/day)
800	69	80	781	1.88
1000	63	120	909	2.18
1200	68	160	1132	2.72

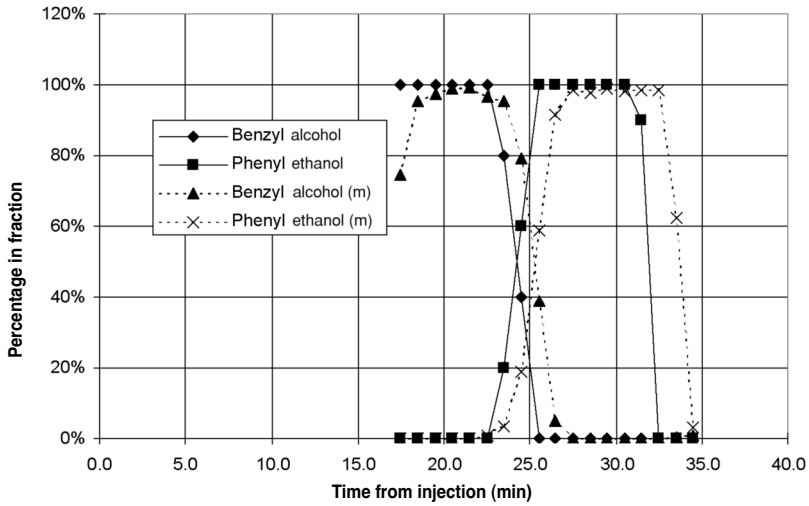

Figure 7. Theoretical versus measured crossover (1000 rpm, 20 mL/min, 100-mL sample).

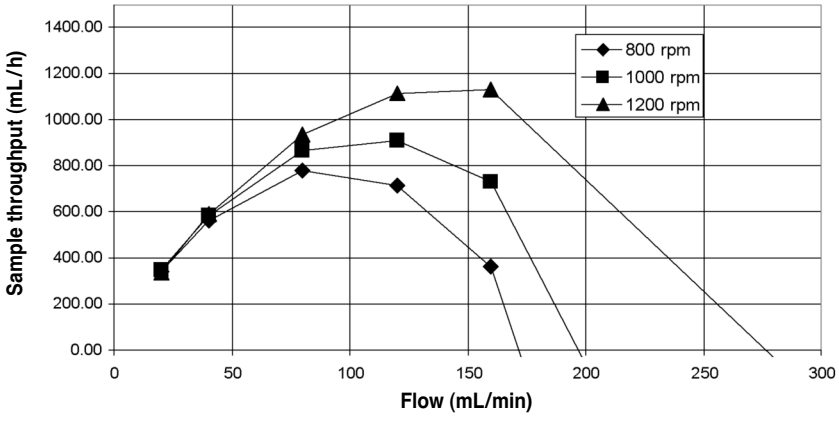

Figure 8. Variation of sample throughput with flow rate.

Engineering for granting a travel award to Philip Wood for his presentation at this conference.

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